AAPTOSAMINE, A NEW 5,8-DIAZABENZ[*cd*]AZULENE ALKALOID FROM THE CARIBBEAN SPONGE *AAPTOS AAPTOS*. AN UNPRECEDENTED BASE-CATALYZED REARRANGEMENT OF 9-DEMETHYLOXYAAPTAMINE

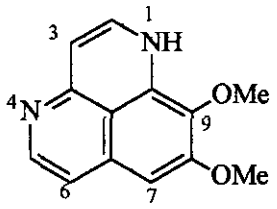
Winston F. Tinto

Laboratory of Bioorganic Chemistry, Department of Biological and Chemical Sciences, University of the West Indies, Cave Hill Campus, P.O. Box 64, Bridgetown, Barbados, West Indies

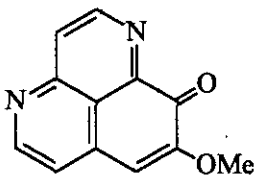
Abstract - A new 5,8-diazabenz[*cd*]azulene alkaloid, aaptosamine, was isolated from the marine sponge *Aaptos aaptos* collected in the southern Caribbean. The structure was determined by 2D NMR spectroscopy using HMQC and HMBC experiments. The known alkaloid 9-demethyloxyaaptamine which was isolated from an earlier collection of *A. aaptos*, underwent an unprecedented base-catalyzed rearrangement to give a new furanonaphthyridine derivative.

The marine sponge *Aaptos aaptos* (Class Demospongiae) collected in Okinawa, has been the subject of a number of chemical investigations wherein a small group of biologically active benzo[*de*][1,6]naphthyridine alkaloids including aaptamine (**1**) and 9-demethyloxyaaptamine (**2**), have been isolated.[1-4] The structure of aaptamine (**1**) was subsequently confirmed by total synthesis.[5,6] More recently, a new cytotoxic 5,8-diazabenz[*cd*]azulene (**3**) was isolated from a Red Sea collection of *A. aaptos* and its structure assigned by 2D NMR spectroscopy.[7] We have investigated the extracts of *A. aaptos* collected off Macqueripe Bay,Trinidad and report here the isolation and characterization of a new 5,8-diazabenz[*cd*]azulene alkaloid, designated aaptosamine (**4**). An earlier chemical investigation of this sponge from an adjacent area resulted in the isolation of (**2**) as the only alkaloid.[8] 9-Demethyloxyaaptamine (**2**) underwent an unprecedented base-catalyzed rearrangement to yield the new furanonaphthyridine derivative (**5**)

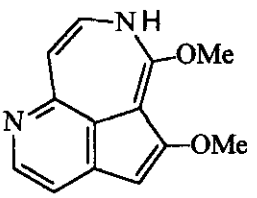
Aaptosamine (**4**), was isolated as an orange gum and had the molecular formula, $C_{15}H_{14}N_2O_3$ as determined by HRMS. The IR spectrum had absorptions characteristic of hydroxyl (3595 cm^{-1}) and carbonyl (1710 cm^{-1}) functionalities. The ^1H NMR spectrum had resonances for a methyl ketone at δ 2.03 and a methoxy group at



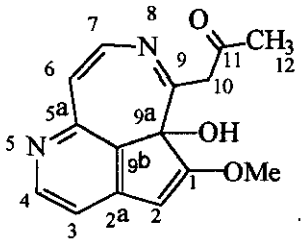
(1)



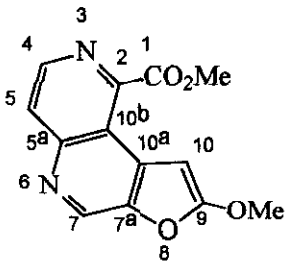
(2)



(3)



(4)



(5)

δ 3.91. In addition, there were low-field resonances due to five aromatic protons at δ 5.93 (s, H-2), δ 7.09 (d, J = 4.4 Hz, H-3), δ 8.83 (d, J = 4.4 Hz, H-4), δ 7.75 (d, J = 5.8 Hz, H-6) and δ 8.68 (d, J = 5.8 Hz, H-7). The presence of a ketone was confirmed by a ^{13}C NMR resonance at δ 206.15 while the methoxy had a resonance at δ 56.08 and a quaternary oxygenated sp^3 carbon at δ 72.2. The HMQC spectrum revealed that the methyl protons at δ 2.03 was directly attached to a carbon at 30.94, while an ABq at δ 3.60 (15.0 Hz) was bonded to a carbon at δ 53.62. The methylene protons at δ 3.60 showed HMBC correlations to the ketone carbon at δ 206.15, the oxygenated carbon at δ 72.20 (C-9a), in addition to carbons at δ 160.22 (C-9) and δ 164.74 (4-bond, C-1). The methoxy protons at δ 3.91 showed HMBC correlations to the carbon at 164.74 (C-1) which indicated that the methoxy group was attached to that carbon. On the other hand, the proton at δ 5.93 (H-2) showed HMBC correlations to the carbons at δ 164.74 (C-1), δ 95.92 (C-2a), δ 117.23 (C-3), δ 72.20 (C-9a) and δ 115.33 (C-9b). The results of these experiments are summarized in Table 1 and led to the structure (**4**) for aaptosamine.

In an earlier chemical investigation of *A. aaptos*, 9-demethyloxyaaptamine (**2**) was isolated[8] and had spectroscopic results in complete accord with literature data.[2] When **2** was stirred with 10% KOH/MeOH for 30 min, followed by careful neutralization with 6 M HCl and subsequent esterification with ethereal diazomethane, the new furanonaphthyridine derivavtive (**5**) was obtained. Compound (**5**) was recrystallized

Table 1. NMR Characteristics of Aaptosamine (**4**) at 500 MHz in $CDCl_3$.

Position	δ_C	δ_H (J_{HH}/Hz)	HMBC
1	164.74	----	----
2	95.92	5.93 (s)	C-1, C-2a, C-3, C-9a, C-9b
2a	140.61	----	----
3	117.23	7.09 (4.4)	C-2, C-4, C-9b
4	155 95	8.83 (4.4)	C-2a, C-3, C-5a
5	(N)		
5a	149.62	----	----
6	121.70	7.75 (5.8)	C-5a, C-7, C9b
7	146.72	8.68 (5.8)	C-6, C-5a, C-9
8	(N)		
9	160.22	----	----
9a	72.20	----	----
9b	115.33	----	----
10	53.62	3.60 (ABq,15.0)	C-9, C-9a, C-11
11	206.15	----	----
12	30.94	2.03 (s)	C-10, C-11
OCH_3	56.08	3.91 (s)	C-1
OH	----	4.24 (br s)	

from chloroform-methanol as colourless prisms, mp 192-193°C. The molecular formula, $C_{13}H_{10}N_2O_4$, was established by HRMS. The IR spectrum had a characteristic ester absorption at 1740 cm^{-1}. The ^1H NMR spectrum had resonances at δ 8.55 (1H, d, J = 5.6 Hz, H-4) and δ 7.73 (1H, d, J = 5.6 Hz, H-5) due to the presence of an aromatic AB spin system. Two isolated aromatic protons had resonances at δ 8.27 (s, H-7) and δ 7.10 (s, H-10). Two resonances at δ 4.15 (3H, s) and δ 4.16 (3H, s) were attributed to two methoxy groups. The IR band at 1740 cm^{-1} coupled with a ^{13}C signal at δ 168.0, indicated that one of the methoxy groups existed as a methoxycarbonyl function. This was supported by an ion at *m/z* 200 (M^+-$C_2H_2O_2$) in the MS spectrum. Signals for ten aromatic carbons were also evident in the ^{13}C NMR spectrum. On the basis of the foregoing evidence, structure (**5**) is proposed for the furanonaphthyridine derivative. Compound (**5**) might have arisen by base-catalyzed cleavage of the C-8/C-9 bond followed by condensation of C-8 with an oxygenated C-6. The oxygen at C-6 would have been the result of aereal oxygenation. The isolation of two different alkaloids from *A. aaptos*, at different times and at different locations might be due to seasonal variations.[8] This phenomenon was also observed in the case of Red Sea specimens of this same organism.[7]

EXPERIMENTAL

The IR spectra were recorded on a Perkin-Elmer 1725X FT-IR spectrophotometer. UV specta were recorded on a Hewlett-Packard 8452A spectrophotometer in MeOH solutions. NMR spectra were recorded on a Varian XL300 or Varian Unity 500 spectrometer in $CDCl_3$ solutions with TMS as the internal standard. MS were recorded on a VG 70-24S mass spectrometer operating at 70 eV.

Animal material The sponges were collected off Rust Bay, Northwestern peninsula, Trinidad in July 1996 and identified by Mr Richard Hubbard, Institute of Marine Affairs, Trinidad and Tobago, where a voucher specimen was deposited. An earlier collection from which 9-demethyloxyaaptamine was isolated, was made in April 1984 from an adjacent area.

Extraction and Isolation The sponge (161 g, wet weight) was extracted with acetone (500 mL) for 72 h at 28°C and the solvent evaporated to give a bark brown extract (3.3 g). The extract was chromatographed on silica gel using $CHCl_3$/MeOH (95:5) as eluent to give fifteen fractions. Fractions 9-12 (0.15 g) were combined and separated by preparative TLC using the same solvent mixture as above to give aaptosamine **4** (27 mg).

Aaptosamine (4): Orange gum; IR (film) 3342, 1714, 1645, 1596, 1578 cm^{-1}; UV (MeOH) 240, 336 nm (log ϵ 4.01, 3.93) , EIMS *m/z* (rel. int.) 270 (M^+, 10), 213 (100), 212 (62), 183 (44), 170 (29), 154 (52), 142 (11), 127 (18), 114 (20); HRMS: [M^+] 270.1002 ($C_{15}H_{14}N_2O_3$ requires 270.1004).

Furanonaphthyridine (5): 9-Demethyloxyaaptamine (**2**) (50 mg) was dissolved in 10% KOH/MeOH (10 mL) and stirred at 30°C for 30 min. The reaction mixture was acidified with 6M HCl and the solvent evaporated. The residue was dissolved in MeOH (2 mL) and ethereal diazomethane was added. The solvent was evaporated, the residue triturated with $CHCl_3$ (10 mL) and the $CHCl_3$ evaporated to give a brown residue (10 mg). The residue was separated by preparative TLC using hexane/EtOAc (3:2) to give **5** (3 mg, 4.9 %).

Compound **5**, colourless prisms, mp 192-193°C; IR (film) 1740, 1630, 1580, 1500 , 1000 cm^{-1}; UV (MeOH) 236, 285, 295, 305, 320 nm (log ϵ 4.58, 3.63, 3.70, 3.69, 3.65); ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (1H, d, J = 5.6 Hz, H-4), 8.27 (1H, s, H-7), 7.73 (1H, d, J = 5.6 Hz, H-5), 7.10 (1H, s, H-10), 4.16 (3H, s, CO_2CH_3), 4.15 (3H, s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0 (C-1), 152.3 (C-7), 149.0 (C-9), 148.6 (C- 2a), 142.7 (C-4), 140.7 (C-2), 136.8 (C-10a), 136.0 (C-7a), 121.7 (C-10b), 113.8 (C-5), 102.8 (C-10), 56.8 (OCH_3), 53.2 (CO_2CH_3); EIMS *m/z* (rel. int.) 258 (M^+, 69), 227 (19), 200 (100), 199 (90), 184 (26), 171 (31) 157 (21), 142 (25), 128 (45), 114 (18); HRMS: [M^+] 258.0650 ($C_{13}H_{10}N_2O_4$ requires 258.0641).

ACKNOWLEDGMENTS

The author wishes to thank Professor William F. Reynolds, University of Toronto, for recording the NMR spectra of aaptosamine and Mr. Richard Hubbard, Institute of Marine Affairs, Trinidad and Tobago, for the collection and identification of the sponges.

REFERENCES

1. H. Nakamura, Y. Kobayashi, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, 1982, **23**, 5555.
2. H. Nakamura, Y. Kobayashi, Y. Ohizumi, and Y. Hirata, *J. Chem. Soc., Perkin Trans. I*, 1987, 173.
3. Y. Ohizumi, H Kajiwara, H. Nakamura, and J. Kobayashi, *J. Pharm. Pharmacol.*, 1984, **36**, 785.
4. Y. Kashman, A. Rudi, S. Hirsch, S. Isaacs, D. Green, D Blasberger, and S. Carmely, *New J. Chem.*, 1990, **14**, 729.
5. J. C. Pelletier and M. P. Cava, *Tetrahedron Lett.*, 1985, **26**, 1259.
6. T. R. Kelly and M. P. Maguire, *Tetrahedron*, 1985, **41**, 3033.
7. A. Rudi and Y. Kashman, *Tetrahedron Lett.*, 1993, **34**, 4683.
8. W. F. Tinto, 'Characterization of Secondary Metabolites fron Caribbean Gorgonians and Sponges,' Ph.D. Thesis, The University of the West Indies, 1986.

Received, 12th June, 1998